# The effects of sucrose abstinence on motivated responding and nucleus accumbens cellular signaling

Joshua Lacey Jones

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Approved by:

Advisor: Dr. Regina M. Carelli

Committee member: Dr. Todd E. Thiele

Committee member: Dr. Rita Fuchs-Lokensgard

## ABSTRACT

Joshua L. Jones: The effects of sucrose abstinence on motivated responding and nucleus accumbens cellular signaling
(Under the direction of Dr. Regina M. Carelli)

A critical feature of cocaine addiction is the strong propensity to relapse following periods of abstinence. Here, we examined whether abstinence from sucrose self-administration in rats altered behavioral responding and nucleus accumbens (NAc) cell firing in a manner similar to that observed following cocaine abstinence. Rats were trained to self-administer sucrose, and then underwent either a 0, 7 or 30 day period of abstinence. Electrophysiological recording procedures were used to examine NAc activity during resumption of sucrose self-administration. Results showed no increase in sucrose-seeking or changes in cell firing of NAc neurons following any abstinence period. Furthermore, in a separate group of animals trained under identical conditions, motivated responding was assessed during extinction and cue-induced reinstatement tasks. No evidence was obtained for any alterations in sucrose-seeking across abstinence conditions. Taken together, these results provide insight about the neural circuitry of natural and drug rewards, and relapse behavior.

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## TABLE OF CONTENTS

LIST OF FIGURES ……..vii

Chapter

I. INTRODUCTION ……1

Reinstatement and relapse to drug-seeking…….1

The role of the nucleus accumbens in reinstatement and relapse. …….3

Neurophysiology of the nucleus accumbens. ……5

II. METHODS ……7

Animals ……..7

Surgery and Sucrose self-administration……..……..7

Electrophysiological recordings ……9

Data analysis ……....10

Histology ……..12

Behavioral extinction test sessions.....……..……13

III. RESULTS ……...…15

Self administration behavior following sucrose abstinence…...…..……..15

NAc cellular response profiles following sucrose abstinence……...……..……15

Behavioral responding during extinction, cue-induced reinstatement and resumption of sucrose self-administration ……...……17

Anatomic distribution……..……..…18

IV. DISCUSSION ……..19

REFERENCES……..29

## LIST OF FIGURES

Figure

1. Behavioral performance during resumption to self-administration.....……...24

2. Neural firing of NAc neurons during resumption to self-administration……..……..…..25

3. Cue-evoked response profiles of NAc neurons……..…….26

4. Behavioral performance during extinction and reinstatement…..…….…….27

5. Anatomical distribution of electrode placements and cell response types……...28

## ABBREVIATIONS

| | |
|---|---|
| DA | Dopamine |
| DSP | Digital signal processor |
| IV | Intravenous infusion |
| NAc | Nucleus accumbens |
| PCA | Principal components analysis |
| PEH | Peri-event histogram |
| S:B | Signal-to-baseline |
| VTA | Ventral tegmental area |

# CHAPTER 1

## INTRODUCTION

One of the most debilitating characteristics of cocaine addiction in humans is the high susceptibility to relapse, even following prolonged periods of abstinence (Gawin 1991; O'Brien 1997). This relapse, occurring months to years after use, is most often triggered by environmental cues and contexts that have come to be associated with previous drug taking. Recent studies have shown that environmental stimuli associated with a single exposure to cocaine are able to elicit long-lasting drug seeking that can persist for up to a year (Ciccocioppo, Sanna et al. 2001; Ciccocioppo, Martin-Fardon et al. 2004). As such, addiction research has utilized animal models of relapse that have allowed researchers to objectively examine behaviors associated with chronic self-administration of drugs and the transition to compulsive drug-seeking (Wolffgramm and Heyne 1995; Ahmed and Koob 1998; Heyne and Wolffgramm 1998; Deroche-Gamonet, Belin et al. 2004; Vanderschuren and Everitt 2004; Vanderschuren and Everitt 2005). Furthermore, application of these animals model procedures have allowed for a systematic examination of relapse to drug seeking behavior following abstinence (for review see (McFarland and Kalivas 2001; Shalev, Grimm et al. 2002; Shaham, Shalev et al. 2003; See 2005)).

### *Reinstatement and relapse to drug seeking*

Animal models of relapse that utilize extinction and reinstatement procedures have provided objective and quantifiable measures of drug-seeking behaviors that resemble the

human condition. In these models, animals are trained to self-administer drugs by performing an operand for an intravenous (IV) infusion of the drug (de Wit and Stewart 1981). Following self-administration training, animals then undergo a period of extinction training where responses on the previous operand have no programmed consequences. Animals are then tested for their ability to reinstate drug-seeking behavior following presentation of a priming stimulus. This priming stimulus can either be re-exposure to the drug, a previously associated cue, or a stressor. The magnitude of the increased responding on the previous operand is the measure of drug-seeking behavior. This is the general form of the reinstatement model, yet there are variations of this protocol in which extinction and relapse can be applied in either between or within session designs (for review (Shalev, Grimm et al. 2002; Kalivas and McFarland 2003; Shaham, Shalev et al. 2003).

The reinstatement model has become a well established means by which to examine the behavioral and motivational shifts associated with abstinence. An early study examined the time-dependent changes in cocaine seeking behavior and dopamine levels following cocaine withdrawal (Tran-Nguyen, Fuchs et al. 1998). In this study, rats were trained to self-administer cocaine and underwent a period of 1 day, 1 week, or 1 month of forced abstinence. Extracellular dopamine and behavioral responding was measured during baseline, extinction, cue and cocaine reinstatement of drug-seeking behaviors. These authors found that cocaine-seeking behavior was intensified following 1 month of abstinence (Tran-Nguyen, Fuchs et al. 1998). Furthermore, DA levels showed corresponding increases in the amygdala following the 1 month withdrawal period. This was the first study that showed increased motivational responding for cocaine following periods of abstinence, and also implicated the dopaminergic system in relapse (Tran-Nguyen, Fuchs et al. 1998).

This study demonstrated that there are shifts in motivational states following abstinence that resemble increased craving in humans and several reports ensued that further characterized and described these behavioral changes (Grimm, Hope et al. 2001; Shalev, Morales et al. 2001; Lu, Grimm et al. 2004; Lu, Grimm et al. 2004). These studies demonstrated similar increases in motivated responding following periods of forced abstinence. Specifically, there were marked increases in extinction, cue-induced and cocaine-induced behavioral responding for abstinence periods as long as six months (Lu, Grimm et al. 2004), as well as for both short (2 hours/day) and long-access (6 hours/day) training periods (Lu, Grimm et al. 2004). Other studies have demonstrated that even following only a single exposure to cocaine self-administration, cocaine-associated stimuli elicit reward-seeking behavior that persists up to one year (Ciccocioppo, Martin-Fardon et al. 2004).

Furthermore, similar phenomena have been observed following periods of abstinence from natural rewards. Using similar behavioral paradigms, aspects of extinction and cue-induced responding are increased following 1 week, 2 weeks or 1 month abstinence from sucrose self-administration (Grimm, Shaham et al. 2002; Lu, Grimm et al. 2003; Lu, Grimm et al. 2004; Grimm, Fyall et al. 2005). However, recent evidence suggests that the amount of exposure and self-administration procedure may determine the degree of the motivational shift (Avena, Long et al. 2005; Jones, Wheeler et al. 2007). Together, these studies suggest that craving 'incubates' as a result of abstinence for drugs of abuse, and under certain circumstances for natural appetitive rewards (Grimm, Hope et al. 2001).

***The role of the nucleus accumbens in reinstatement and relapse***

The nucleus accumbens (NAc) has been shown to be intricately involved in natural reward processes such as food, sex and adaptive social behaviors (Hernandez and Hoebel 1988; Wise and Rompre 1989; Damsma, Pfaus et al. 1992; Johnson, Parente et al. 1996; Kelley, Bless et al. 1996; Becker, Rudick et al. 2001; Aragona, Liu et al. 2003; Kelley 2004; Roitman, Stuber et al. 2004; Aragona, Liu et al. 2006) and drug addiction (Berridge and Robinson 1998; Self 1998; Wise 1998; Robinson and Berridge 2001; Di Chiara 2002; Adinoff 2004; Kelley 2004). As such, the NAc is ideally situated to influence and process information related to drug-stimulus associations and relapse to drug-seeking (Cornish and Kalivas 2000; McFarland and Kalivas 2001; Kalivas and McFarland 2003; Peters and Kalivas 2006). Indeed, several lines of research converge in suggesting that the NAc mediates goal-directed behaviors for both drug and natural (i.e., water, sucrose, & food) rewards (Carelli and Deadwyler 1994; Wise 1998; Kelley 1999; Carelli, Ijames et al. 2000; Robbins and Everitt 2002; Di Chiara, Bassareo et al. 2004; Roitman, Stuber et al. 2004). Moreover, conditioned cues associated with primary reinforcement can evoke patterned response profiles among NAc neurons and elicit dopamine release in this structure (Carelli 2000; Phillips, Stuber et al. 2003; Nicola, Yun et al. 2004; Roitman, Wheeler et al. 2005).

Prolonged cocaine self-administration results in long-lasting neuroadaptations within reward-related neural systems, and these neuroadaptations likely underlie the motivational changes associated with relapse and craving (Kalivas, Pierce et al. 1998; Self and Nestler 1998; Nestler 2004). Furthermore, it has been suggested that similar molecular mechanisms may underlie goal-directed behaviors for drug and natural rewards, and that study of the neural circuitry mediating the seeking of natural rewards may advance our understanding of the neural basis of drug addiction (Kelley and Berridge 2002). Recent studies examining

abstinence-induced changes within these regions reported numerous neuroadaptations in the NAc following periods of cocaine abstinence (Baker, McFarland et al. 2003; Kalivas, McFarland et al. 2003), (Toda, McGinty et al. 2002). Importantly, several studies have suggested that neuroadaptations are more profound after abstinence from drug rewards compared to natural rewards. For example, structural changes in NAc medium spiny neurons have been observed following 1 month removal of cocaine, but not from food (Robinson, Gorny et al. 2001). Furthermore, 1-month abstinence from cocaine or sucrose self-administration results in various molecular neuroadaptations within the NAc and ventral tegmental area that are specific to cocaine and not sucrose (Lu, Grimm et al. 2003).

***Neurophysiology of the NAc***

Electrophysiological recordings of NAc neurons in rodents engaged in cocaine and sucrose self-administration have provided important insight into cellular mechanisms that underlie reward-seeking behaviors. Those studies revealed that, in well-trained animals, a subset of NAc neurons exhibit patterned response profiles, characterized by changes (increases and/or decreases) in firing rate time-locked to the reinforced response for either cocaine or sucrose (Carelli, King et al. 1993; Peoples and West 1996; Carelli 2002; Taha and Fields 2006). These time-locked changes appear to reflect certain associative aspects of the self-administration task (Carelli, King et al. 1993; Carelli 2002; Nicola, Yun et al. 2004). Remarkably, recent findings from our laboratory have shown a significant 2-fold increase in the incidence of NAc patterned discharges following 1 month abstinence from cocaine self-administration coincident with an increased motivation for the drug (Hollander and Carelli 2005). In addition, NAc neurons are more activated by cocaine-associated cues following 1-

month abstinence and this activation is linked to higher rates of cocaine-seeking behavior (Hollander and Carelli 2007).

Here, we examined whether a similar increased incidence in NAc neurons that encode information about goal-directed behavior for a natural (sucrose) reward would be observed following abstinence. Specifically, animals were trained to self-administer sucrose, then NAc cell firing was recorded during resumption of sucrose self-administration following either 1 (control), 7 or 30 days of experimenter-imposed abstinence. Additionally, another set of animals was used to examine behavioral responding, after identical training and abstinence periods, during an extinction and cue-induced reinstatement paradigm. Compared with our prior work with cocaine (Hollander and Carelli 2005; Hollander and Carelli 2007), the present studies allowed an examination of whether similar cellular neuroadapations exist following abstinence from sucrose self-administration in the NAc, thereby providing new information concerning the effects of abstinence on behavioral and neural responding during goal-directed actions for drugs versus 'natural' rewards.

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# CHAPTER 2

## METHODS

### *Animals*

Male, Sprague Dawley rats (n=39, Harlan Sprague Dawley, Indianapolis, IN) aged 90-120 d and weighing 275-350 gm were used as subjects and individually housed with a 12:12 light:dark cycle. Bodyweights were maintained at no less than 85% of pre-experimental levels by water restriction (25-45 ml of water each day). This regimen was in place for the duration of behavioral testing, except during the post-operative recovery period when water was given *ad libitum*. All procedures were approved by the University of North Carolina Institutional Animal Care and Use Committee.

### *Surgery and Sucrose Self-Administration*

A subset of animals (n=22) were prepared for extracellular recording in the NAc via implantation of microwire electrode arrays. Each array was custom-designed (consisting of eight microwires each with 50μm diameter and arranged in a 2 x 4 configuration) and purchased from a commercial source (NB Labs, Denison, TX). Arrays were permanently implanted bilaterally into the NAc core or shell (AP: +1.7mm, ML:+/-1.3 and +/-0.8mm, DV: -6.2mm from brain, relative to bregma, level skull; (Paxinos 2005)). All surgical procedures were approved by the UNC Institutional Animal Care and Use Committee (IACUC) in concordance with the NIH Guide for the Care and Use of Animals.

One week following surgery, animals were trained to self-administer sucrose (0.3 M) during daily 1 h sessions conducted in a 43 x 43 x 53 cm Plexiglas chamber (Med. Associates, Inc., St Albans, VT) housed within a commercial sound-attenuated cubicle (Fibrocrete, Inc., Crandall, GA). Here, we used a similar version of a paradigm described previously (Grimm, Fyall et al. 2005). Importantly, we used the same reinforcer (0.3M sucrose), the same total number of reinforcers per training session (30), and the same abstinence conditions (1, 7 and 30 days), However, slight modifications were made to both the training and extinction/reinstatement (see below) designs to allow for neurophysiological assessments. First, during training, animals were allowed to lever press for a total of 30 reinforcers during a single 1 h session, whereas previous work (Grimm, Fyall et al. 2005) divided the 30 reinforcers into two forced 1 h sessions, where the animal remained in the dark, with no presented stimuli once the initial 15 trials were finished. The beginning of the self-administration session was signaled by the onset of a cue-light positioned 6.5 cm above an active lever that extended into the chamber. Concurrently an inactive lever was inserted into the chamber that remained extended throughout the session. Operant responses on the active lever were reinforced on an FR1 schedule with liquid sucrose delivery (0.3ml/infusion over 1.5 s) into a drinking receptacle located between the levers via a computer controlled syringe pump (Model PHM-100, Med.). Initiation of sucrose delivery was accompanied by termination of the cue-light, simultaneous onset of a tone (67 db, 1 kHz, 1 s) and retraction of the active lever. The active lever was re-extended into the chamber, paired with the cue-light, after a 20 sec time-out. Responses on the inactive lever resulted in no programmed consequences. All animals were trained over a seven day period (30 presses/session). Sucrose self-administration sessions were limited to 30 trials to remain similar to behavioral

responses during cocaine self-administration, reported previously (Hollander and Carelli 2005). Animals were tethered during the final two days of training to habituate them for the upcoming recording session.

Animals were then randomly divided into three groups (Control, 7 Day or 30 Day) and NAc cell firing was recorded during a single sucrose self-administration test session. Each animal received only one electrophysiological recording session, and this occurred following the specified abstinence period. The control group (n=7 rats) underwent no experimenter imposed abstinence period from sucrose, beyond the normal 24 h period between sessions, thus controlling for duration of abstinence. This 24 h period group served as the control for the remaining groups, in which animals underwent either 7 (n=7) or 30 days (n=8) abstinence, where access to sucrose was interrupted (animals remained in home cages). Following abstinence, animals were placed back in the experimental chamber and NAc neuronal activity was recorded during completion of a single sucrose self-administration session as described above.

***Electrophysiological Recordings***

Electrophysiological procedures have been described in detail previously (Carelli and Deadwyler 1994; Carelli, Ijames et al. 2000) Briefly, before the start of each session, the subject was connected to a flexible recording cable attached to a commutator (Med Associates Inc., St Albans, VT), which allowed virtually unrestrained movement within the chamber. NAc activity was recorded differentially between each active and the inactive (reference) electrode from the permanently implanted microwires. The inactive electrode was examined before the start of the session to verify the absence of neuronal spike activity and served as the differential electrode for other electrodes with cell activity. Online isolation

and discrimination of neuronal activity was accomplished using a neurophysiological system commercially available (multi-channel acquisition processor, MAP System, Plexon, Dallas, TX, USA). Multiple window-discrimination modules and high-speed analog-to-digital (A/D) signal processing in conjunction with computer software enabled isolation of neuronal signals based on waveform analysis. The neurophysiological system incorporated an array of digital signal processors (DSPs) for continuous spike recognition. The DSPs provided a continuous parallel digital output of neuronal spike events to a Pentium computer. Another computer controlled behavioral events of the experiment (Med. Associates) and sent digital outputs corresponding to each event to the MAP box to be time stamped along with the neural data. Principal component analysis (PCA) of recorded waveforms was performed prior to each session and aided in the separation of multiple neuronal signals from the same electrode. This sophisticated analysis generates a projection of waveform clusters in a three-dimensional space, enabling manual selection of individual waveforms. Before each session, an individual template made up of many 'sampled' waveforms was created for each cell isolated using PCA. During the behavioral session, waveforms that 'matched' this template were collected as the same neuron. Cell recognition and sorting was finalized after the experiment using the Offline Sorter program (Plexon), when neuronal data were further assessed based on PCA of the waveforms, cell firing characteristics, and interspike intervals.

***Data Analysis***

Neural activity was initially characterized via raster displays and peri-event histograms (PEHs) using commercially available software (Neuroexplorer, Plexon). The PEHs and rasters displayed the activity of each cell relative to sucrose-reinforced lever press responses. NAc cell firing was classified into one of three well-defined types of phasic

neuronal firing patterns that occurred within seconds of the reinforced response, or nonphasic activity. Specifically, phasic responses were characterized by an anticipatory increase in activity preceding the reinforced response (termed type "pre-response" or PR), or an increase immediately following the reinforced response (type "reinforcement-excitation", RFe). A third cell type exhibited decreases in firing surrounding the reinforced response (type "reinforcement-inhibition", RFi). Neurons were classified as nonphasic if they showed no significant change in firing rate relative to the reinforced response. Statistical verification of cell type classification was accomplished in the following manner. First, average firing rates of each neuron were calculated within 1 s time epochs across all 30 trials within a 20 s analysis interval (10 s prior to and 10 s following reinforced responses). Neurons were classified as either type PR, RFe or RFi based upon significant changes (increases or decreases) in firing rate within specific 1 s epochs. Neurons were classified as type PR if they exhibited a significant increase in firing rate within at least one 1 s epoch across the 3 s epochs preceding the response relative to baseline. Neurons were classified as type RFe if they exhibited a significant increase in firing rate within at least one 1 s epoch across the 3 s following the response, relative to baseline. Neurons were classified as type RFi if they exhibited a significant decrease in firing rate within at least one 1 s epoch across the 3 s following the response, relative to baseline. The baseline epoch included -10 to -9 s prior to the reinforced response and did not vary across neurons or sessions. Statistical determination of cell type classification was accomplished using a repeated-measure ANOVA followed by Fisher's LSD *post-hoc* tests to determine which 1 s epochs were significantly different from baseline ($p<0.05$). Cells with extremely low firing rates (<0.5 spikes/s), or relatively high

firing (>10 spikes/s) were likely not medium spiny neurons (Berke, Okatan et al. 2004) and were excluded from statistical analysis (n= 11 cells).

To quantify the strength of each neural correlate as a function of abstinence, signal-to-baseline (S:B) ratios were calculated. Signal was defined as the average peak firing rate within the 1 s epoch showing the largest significant change, relative to baseline, determined as described above. The baseline epoch included -10 to -9 s prior to the reinforced response. S:B ratios were determined by dividing the signal by the baseline. A value of 1 indicates no change (nonphasic firing), values greater than 1 indicate increases in firing rate and values less than 1 indicate inhibitions.

***Histology***

Following the completion of the last experiment, animals were anesthetized with ketamine hydrochloride (100mg/kg) and xylazine hydrochloride (20mg/kg), and a 13.5 μA current was passed for 5 s through all recording wires. Animals were perfused with 10% formalin and 3% potassium ferracyanide, then brains were removed, blocked and sectioned (40 μm) throughout the rostral-caudal extent of the NAc. Sections were stained for thionin to aid with identification of structures and location of the blue dot reaction product corresponding to the location of the marked electrode tip (Carelli, Ijames et al. 2000) To reconstruct electrode positions, serial sections were examined under a light microscope and the locations of all marked electrode tips were plotted for all subjects on coronal sections taken from the stereotaxic atlas of Paxinos and Watson (Paxinos 2005) Only neurons recorded from wires positioned in the NAc were used in the present study. Chi-square analyses were completed to determine whether there were significant differences in the

number of phasic neurons recorded for each condition across subregions (core vs shell) of the NAc.

***Behavioral Extinction Test Sessions***

Another group of rats (n=17) also underwent behavioral self-administration training followed by a single behavioral test session (no electrophysiology). Following training they were divided into three groups; Control (n=5 rats), 7 Day (n=6), 30 Day (n=6) and underwent abstinence periods as described for the previous study. Following abstinence, animals were placed back in the experimental chamber and lever press responding was measured during extinction/reinstatement tests conducted during a single test session divided into three phases; extinction, cue-induced responding, and sucrose self-administration. These sessions were modified slightly from previous studies (Grimm, Shaham et al. 2002; Grimm, Fyall et al. 2005). Specifically, animals underwent only a single extinction session rather than six consecutive hour-long sessions. However, since differences in motivated responding were reported to exist for sucrose in the initial 1 hour extinction period (Grimm, Shaham et al. 2002; Grimm, Manaois et al. 2007), we shortened the extinction session and allowed the extinction period to be determined by the animals responding (see below), rather than measuring the spontaneous recovery that occurs following each time-out period.

The beginning of the extinction phase was signaled by the extension of both the active and inactive levers into the chamber. Unlike the previous training sessions, the cue-light was not illuminated during this test phase. Operant responses on the active lever had no programmed consequences (i.e. did not result in liquid sucrose delivery or conditioned-cue delivery). The extinction phase ended when the animal failed to respond on the active lever for a 30 min period. At the end of this phase, both levers retracted for a 10 min period.

The second phase consisted of cue-induced reinstatement of operant responding. The beginning of the session was signaled by the onset of the cue-light positioned 6.5 cm above the active lever and the extension of both active and inactive levers. A single, non-contingent presentation of the conditioned reinforcer complex (cue-light off, tone-on, lever retraction) was presented at the beginning of the session with no presentation of sucrose. Any further operant responses on the active lever were reinforced with only the conditioned reinforcer complex on an FR1 schedule (no sucrose). Responses on the inactive lever resulted in no programmed consequences. The cue-induced reinstatement phase ended when the animal failed to respond on the active lever for a 30 min period. At the end of this phase, both levers retracted from the chamber and all lights were extinguished for a 10 min period.

Finally, the third phase involved resumption of sucrose self-administration. The beginning of the self-administration session was signaled by the onset of a cue-light positioned 6.5 cm above an active lever and the extension of both active and inactive levers. Operant responses on the active lever were reinforced on the same FR1 schedule used in the training sessions; each active response resulted in sucrose delivery paired with the conditioned reinforcer complex followed by a 20 s time-out period. Responses on the inactive lever resulted in no programmed consequences.

# CHAPTER 3

## RESULTS

### *Self-administration behavior following sucrose abstinence*

Behavioral records of lever press responses during sucrose self-administration for a representative control rat and rats that underwent either 7 or 30 days of sucrose abstinence are illustrated in Figure 1a. No clear differences were observed in responding across abstinence conditions. Likewise, similar behavioral profiles were observed across all animals as a function of abstinence condition. A one-way ANOVA revealed no significant difference in the average number of presses between the conditions across all rats ($F=0.64$, $p>0.53$; Figure 1b). Furthermore, no significant differences across groups were observed in latency to first press ($F=0.5074$, $p>0.61$, Figure 1c), or total session length ($F=1.852$, $p>0.18$, Figure 1d), indicating that abstinence did not alter the motivation to obtain sucrose.

### *NAc cellular response profiles following sucrose abstinence*

In total, 199 NAc neurons were recorded during the single self-administration session following abstinence (Control: 66 neurons, 7 Day: 69 neurons, 30 Day: 64 neurons). Of 199 neurons, 88 exhibited one of the three types of well-documented phasic neural response profiles relative to the lever press response for sucrose, illustrated in Figure 2a. Across abstinence conditions, 36 neurons exhibited increased firing within seconds preceding the reinforced response and were classified as type PR (Figure 2a left). Thirteen neurons displayed excitations in the seconds following the reinforced response and classified as type

RFe (Figure 2a center). Finally, 39 neurons displayed inhibitory activity in the seconds before and/or after the reinforced response and were classified as type RFi (Figure 2a right).

An important issue addressed in this report is whether the dynamics of these response profiles shift as a result of experimenter imposed sucrose abstinence, similar to that observed following cocaine abstinence (Hollander and Carelli 2005; Hollander and Carelli 2007). However, a major finding reported here is that following 7 or 30 day abstinence from sucrose self-administration there is no change in the percentage of neurons that exhibit one of the three phasic response profiles (Figure 2b). Specifically, the average percentage of total numbers of cells that exhibited a phasic response (types PR, RFe or RFi) were 45%, 40% and 44% for the Control, 7 Day and 30 Day groups respectively (Figure 2b); a one-way ANOVA revealed that there was no significant difference between the groups as a function of abstinence ($F=0.1492$, $p>0.86$). To illustrate this further, figure 2c shows composite pie charts of the percentage of NAc neurons exhibiting each type of phasic (type PR, RFe or RFi) or nonphasic response profile across abstinence conditions. A two-way ANOVA revealed no change in the occurrence of any of the cell types as function of abstinence condition ($F=0.6378$, $p>0.53$).

To determine whether the strength of the neural responses was altered following abstinence, we calculated signal to baseline (S:B) ratios across abstinence conditions. There were no significant differences in S:B ratios across abstinence conditions for type PR (figure 2d; $F=1.152$), RFe (figure 2e; $F=0.3593$) or RFi (figure 2f; $F=0.5654$) neurons. Importantly, there was also no change in the baseline firing rates across abstinence conditions ($F=0.4743$). Collectively, these findings demonstrate that not only are the total number and percentage of

phasic cells unaltered as a result of experimenter forced abstinence, but the signal strength of individual response profiles does not change.

In addition to response profiles surrounding the operant response, we also examined the NAc responses to the re-extension of the lever and cue-light onset. Figure 3 shows the results of this analysis. Figures 3a-c show the example cells that exhibit the brief cue excitations that occur at the cue-onset across the three abstinence conditions. Additionally, figures 3d-f show that average of the cue responses across each of the abstinence conditions, demonstrating the lack of change in NAc response profiles. Figures 3g and h show that neither the mean phasic percentage of cells or the strength of signal (S:B) change as a function of abstinence.

***Behavioral responding during extinction, cue-induced reinstatement and resumption of sucrose self-administration***

Our aforementioned findings show that there were no changes in self-administration or NAc cell firing properties as a function of sucrose abstinence. One possible explanation for these negative findings is that animals were not more motivated to respond for sucrose following abstinence in our experimental design. To further examine this possibility, another set of animals were trained to self-administer sucrose as before, and then examined during an extinction/reinstatement test session. Figure 4a shows the average number of presses for all animals across the initial phase of extinction during which active lever press responses did not result in any programmed consequences. A one-way ANOVA of active lever presses during the initial extinction phase revealed no significant difference in the number of presses as a function of abstinence condition ($F=1.272$, $p>0.31$). Likewise, no significant differences in cue-induced lever press responding across conditions were observed during the second

phase in which animals responded for sucrose-associated stimuli only (no sucrose) following a single priming cue ($F=0.1587$, $p>0.85$, Figure 4b). In phase 3, rats resumed sucrose self-administration with no significant change in the time to complete the phase ($F=0.5175$, $p>0.60$, Figure 4c), or in the total number of responses (data not shown). Responding on the inactive lever was negligible across all groups during all phases of the extinction/reinstatement test (data not shown, $F=1.243$, $p>.30$). Collectively, these findings indicate that animals were not more motivated to respond for sucrose following 7 or 30 days of sucrose abstinence.

***Anatomic distribution***

Coronal sections showing the location of microwire electrodes (n=22 rats) from which NAc neurons were recorded for the control, 7 Day and 30 Day abstinence groups are shown in Figure 5. A relatively even distribution of electrode placements was observed within the core and shell (defined by Zahm and Brog, 1992) for the control (45 core, 52 shell), 7 Day (42 core, 45 shell) and 30 Day (52 core, 62 shell) abstinence groups. Chi-square analysis showed that there were no significant differences in the number of electrodes positioned in the core versus shell across abstinence condition ($\chi^2=0.144$, $df=2$; $p>0.05$). Furthermore, abstinence from sucrose does not lead to any alterations in the incidence of phasically active neurons dependent upon the anatomical region of the NAc ($\chi^2=0.689$, $df=2$; $p>0.05$).

# CHAPTER 4

## DISCUSSION

Here we examined neurophysiological and behavioral changes during the resumption of sucrose self-administration following sucrose abstinence. It has been suggested that neuroadaptations of mesolimbic dopamine systems underlie both the increased motivation to seek rewards and the high incidence of relapse following periods of abstinence (Nestler 2004). In this study, we demonstrate that both behavioral and neural responding are unaltered following periods of 7 or 30 days of abstinence from sucrose-seeking. Specifically, we measured NAc cell firing during the resumption to sucrose-seeking and found no significant changes in the NAc encoding of sucrose-related information as a result of abstinence. Furthermore, we found no marked behavioral changes in motivated responding during extinction/reinstatement conditions in rats that underwent identical training and abstinence duration from sucrose-seeking. Collectively, our findings indicate that abstinence from a natural reward such as sucrose may not elicit dramatic increases in motivation to obtain and consume them, or changes in neural coding in the NAc, in the same manner that drug rewards do (Hollander and Carelli 2005; Hollander and Carelli 2007).

A hallmark of human drug addiction is the strong propensity to relapse following periods of abstinence. Previous work has modeled this behavior and shown that rats are more responsive to rewards and reward-related stimuli after periods of experimenter-imposed abstinence (Tran-Nguyen, Fuchs et al. 1998; Grimm, Hope et al. 2001; Grimm, Shaham et al.

2002; Hollander and Carelli 2007). Using extinction and cue-induced reinstatement paradigms, it has been shown that cocaine-seeking behavior becomes greater following 1-month of abstinence in rodent models (Tran-Nguyen, Fuchs et al. 1998). As such, it was proposed that craving 'incubates' over the time course of abstinence or withdrawal from rewards (Grimm, Hope et al. 2001; Grimm, Shaham et al. 2002; Grimm, Lu et al. 2003; Lu, Grimm et al. 2003; Lu, Grimm et al. 2004). Importantly, several of these studies showed that this incubation occurs following not only drug abstinence but also after abstinence from a natural reinforcer such as sucrose (Grimm, Shaham et al. 2002; Grimm, Fyall et al. 2005; Grimm, Manaois et al. 2007). Furthermore, this incubation is not altered by reduced training or pre-exposure to the reward (Grimm, Fyall et al. 2005).

It is interesting then, that the present study provides evidence that experimenter-imposed abstinence from sucrose does not alter the motivation to respond for sucrose or sucrose-related cues. Here, we used a similar training protocol that was successfully used by others to induce increased motivated behavior for sucrose after abstinence (Grimm, Fyall et al. 2005). In other animals trained in the same manner, we show that during sucrose extinction and resumption conditions, rats failed to exhibit a general increase in motivation and did not pursue sucrose or its associated cues at higher rates following abstinence. In fact, the data indicate a trend toward a decrease in motivation following 30 days of abstinence (Fig. 3). These data suggest that the effects of abstinence from a natural reinforcer such as sucrose are not as robust as those seen with cocaine, and may be highly dependent upon the specific experimental conditions. For example, it may be necessary to provide repeated, short periods of sucrose access during training to obtain increased sucrose seeking following abstinence, as reported by Grimm and colleagues (Grimm, Fyall et al. 2005). Alternatively,

increased sucrose seeking following abstinence may be observed when more sucrose trials are permitted during training sessions (Grimm, Manaois et al. 2007). Furthermore, shifts in motivation for sucrose following abstinence may be a result of feeding schedules that induce dependence. In this regard, recent evidence has shown that rats with prolonged (12 h) daily access to sugar show withdrawal symptoms that mimic opioid withdrawal (Colantuoni, Rada et al. 2002), and exhibit enhanced sucrose intake after abstinence. In contrast, rats with only 30 min/day access, did not show withdrawal-like symptoms and actually decreased their responding following abstinence (Avena, Long et al. 2005), in keeping with findings presented here. Additionally, long-access to sucrose (12 hr/day) results in cross-sensitization to amphetamine (Avena and Hoebel 2003) and increased intake of ethanol (Avena, Carrillo et al. 2004).

Given our behavioral findings, it is not surprising then that the incidence of phasic NAc neuronal activity was not altered following periods of experimenter-imposed sucrose abstinence. Specifically, our findings show that sucrose abstinence did not result in changes in either the number or strength of NAc neurons that encode sucrose-seeking behavior. This is in contrast to our prior studies showing that abstinence from cocaine self-administration resulted in a significant 2-fold increase in the incidence of NAc neurons that show phasic response profiles following 1 month cocaine abstinence, correlated to increased motivation for the drug (Hollander and Carelli 2005). Likewise, we recently reported that NAc neurons are more activated by cocaine-associated cues following 1-month abstinence and this activation is linked to higher rates of cocaine-seeking behavior (Hollander and Carelli 2007). The finding that sucrose abstinence does not alter behavioral or neurophysiological responses in the present study indicates that the neurophysiological changes seen in cocaine-abstinent

animals may reflect a motivational shift towards "compulsive" reward-seeking behavior that is prevalent with a highly abused substance such as cocaine, and observed only under specific experimental conditions with a natural reward such as sucrose. In this regard, the finding that there are no changes in NAc cell firing following sucrose abstinence in the present study confirms that neurophysiological changes reported with cocaine (Hollander and Carelli 2005; Hollander and Carelli 2007) truly reflect shifts in the motivational state of the animal and not simply naturally occurring alterations in NAc activity that occur as a result of time.

It has been reported that different anatomic and molecular neuroadaptations are associated with abstinence from drug versus natural rewards. For example, it has been shown that there is increased dendritic branching and spine density of NAc medium spiny neurons following 1-month cocaine abstinence, but not food (Robinson, Gorny et al. 2001). Additionally, specific molecular neuroadaptations are seen in both the NAc and ventral tegmental area following cocaine but not sucrose abstinence, including various alterations in glutamate receptor subunits, PKA activity, tyrosine hydroxylase levels and adenyl cyclase activity (Lu, Grimm et al. 2003). There are also time-dependent increases in brain-derived neurotrophic factor (Grimm, Lu et al. 2003) and immediate early gene expression (Koya, Spijker et al. 2006) in various structures such as the cortex, NAc, VTA and amygdala following cocaine or heroin but not sucrose abstinence. In fact, it has been postulated that these neuroadaptations may underlie the motivational changes associated with relapse and craving that characterize drug addiction (McFarland and Kalivas 2001; Nestler 2004). It then follows that we would expect that NAc activity, as well as activity within other brain regions, would be altered in behavioral paradigms where molecular neuroadaptations are seen.

The absence of increased sucrose seeking following abstinence in the present study indicates that this behavioral result is very sensitive to experimental conditions. It may be the case that alterations in NAc cell firing coupled to increased sucrose seeking can be observed within specific behavioral protocols. For example, if these neurophysiological changes in the NAc are truly a reflection of a motivational shift, such alterations should be seen in experimental designs that elicit a level of 'dependence' with respect to natural rewards (Avena, Long et al. 2005). Future research from this perspective will go far to describe the differential processing of natural and drug rewards, specifically during normal and dysfunctional motivated behavior.

**Figure 1.** Sucrose self-administration did not significantly change across abstinence conditions (A) Behavioral records of three representative rats from each condition show similar response patterns in the first 1000 s of the self-administration session. Across all rats, no significant changes were observed in the average number of lever presses (B), latency to the first press (C) or in the time to complete the self-administration session (D) as a function of abstinence condition. Error bars indicate standard error of the mean.

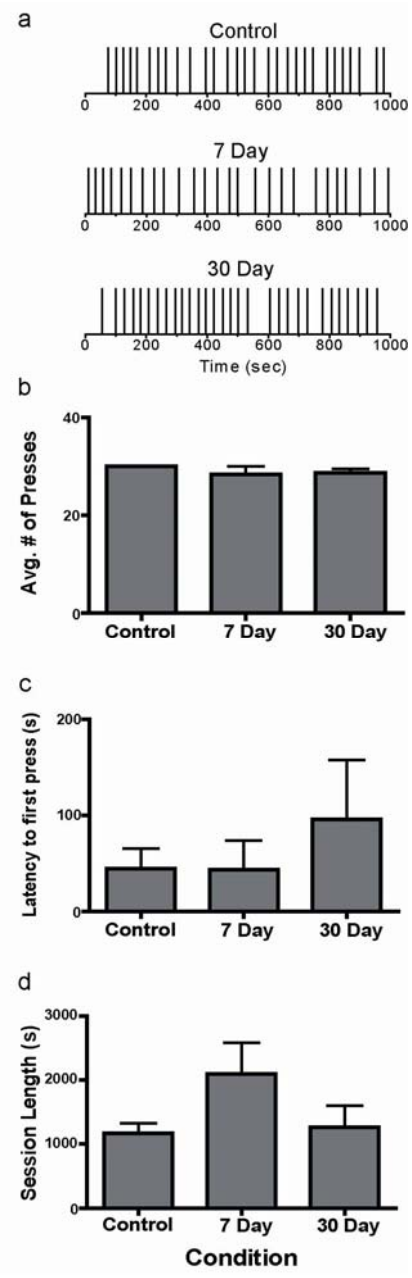


**Figure 2.** No change in the incidence of NAc patterned discharges (types PR, RFe, RFi) following sucrose abstinence. (a) Examples of the three types of well-documented NAc neuronal firing patterns observed relative to the reinforced response for sucrose. Reinforced responses are indicated by R at dashed lines in PEHs. (b) the average percentage of NAc neurons that exhibited patterned discharges (types PR, RFe, RFi) across the three abstinence conditions. (c) piecharts illustrating the distribution of each cell type across abstinence. Signal to baseline ratios as a function of abstinence condition for cell types PR (d), RFe (e), and RFi (f).

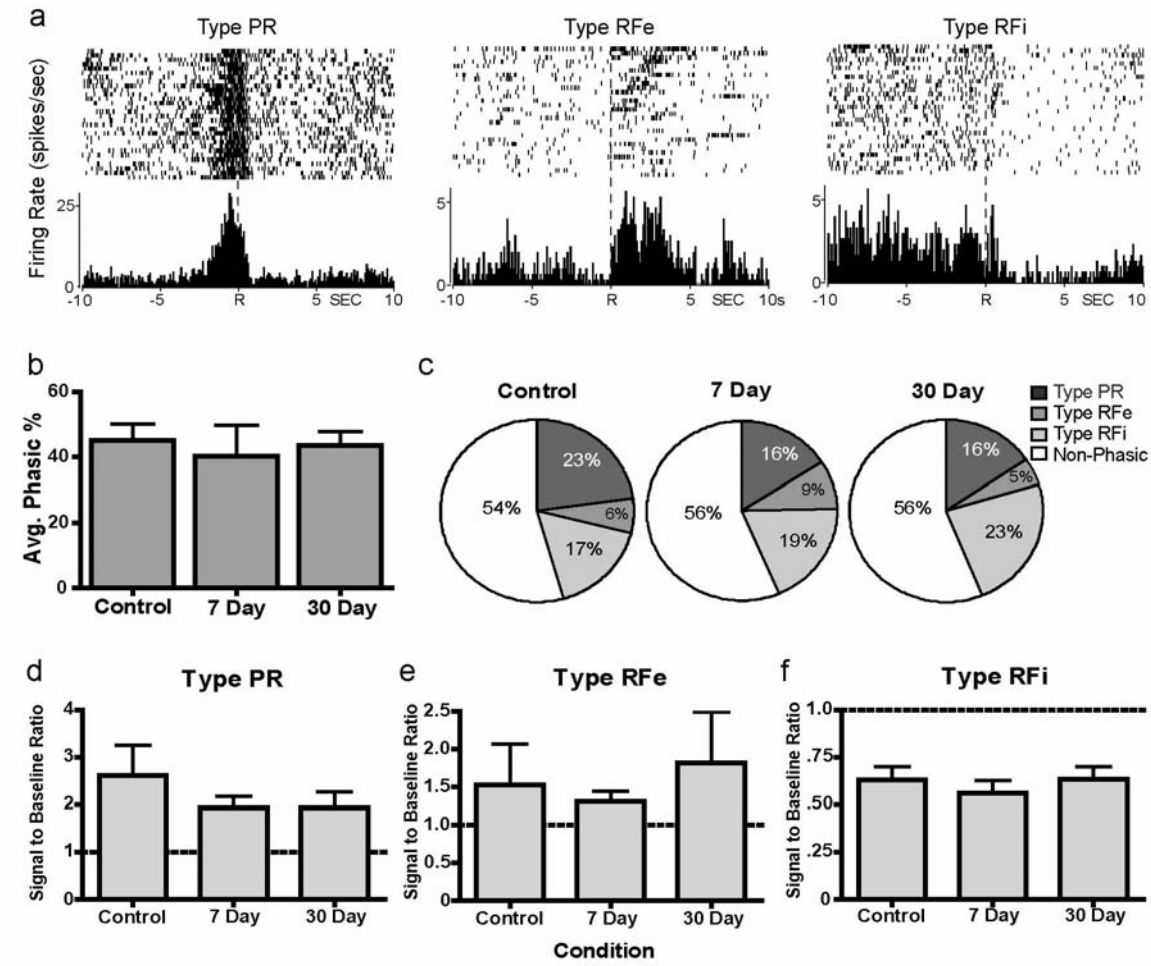


Figure 3. No change in NAc cue responsive neuronal response profiles following sucrose abstinence. (a-c) Examples of the three types of NAc neuronal firing patterns observed relative to the lever extension-cue light discriminative stimulus. Cue onset is indicated by 'cue' in PEHs. (d-f) average traces of neural response profiles across the three abstinence conditions. (g) average percentage of neurons that exhibited cue responsive profiles across abstinence. (h) signal to baseline ratios as a function of abstinence condition for cue responsive cell types.

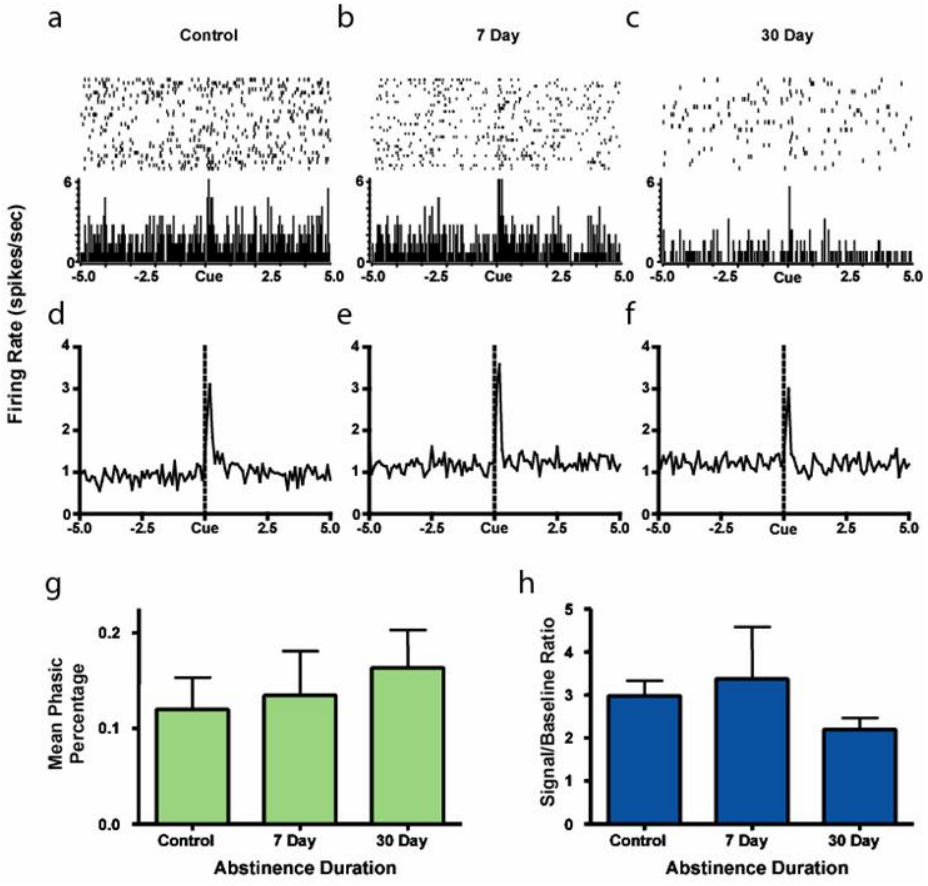


**Figure 4.** Behavioral responding during extinction, cue-induced reinstatement, and resumption of sucrose self-administration was not altered as a function of abstinence. (a) average number of responses on the active lever during the initial extinction phase across abstinence conditions, (b) average number of lever press responses during the cue-induced reinstatement phase across condition, (c) length of self-administration phase following resumption of sucrose self-administration Error bars indicate standard error of the mean.

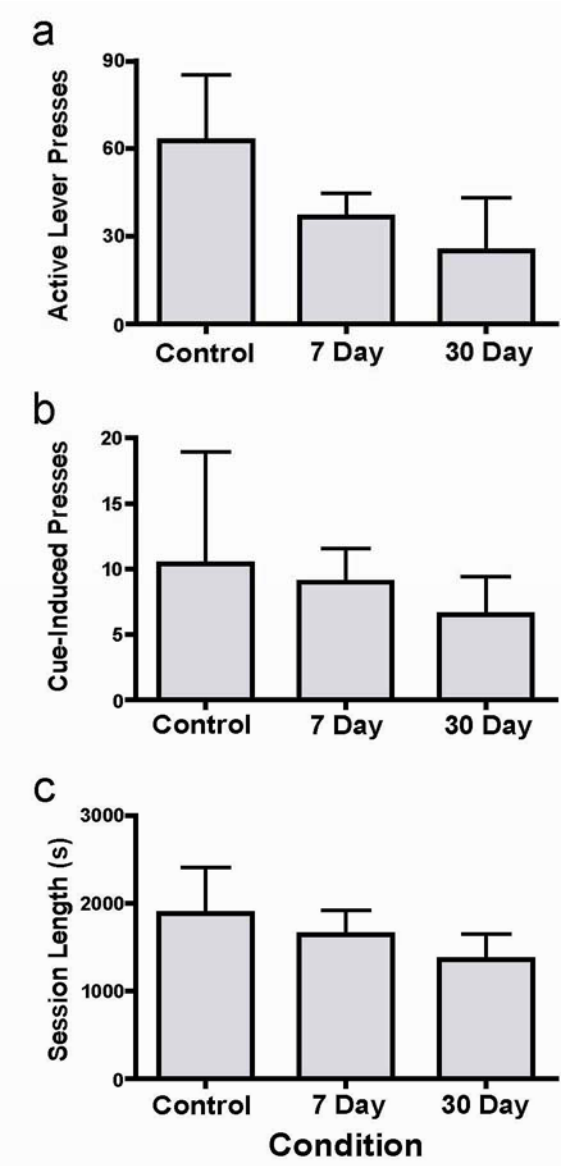
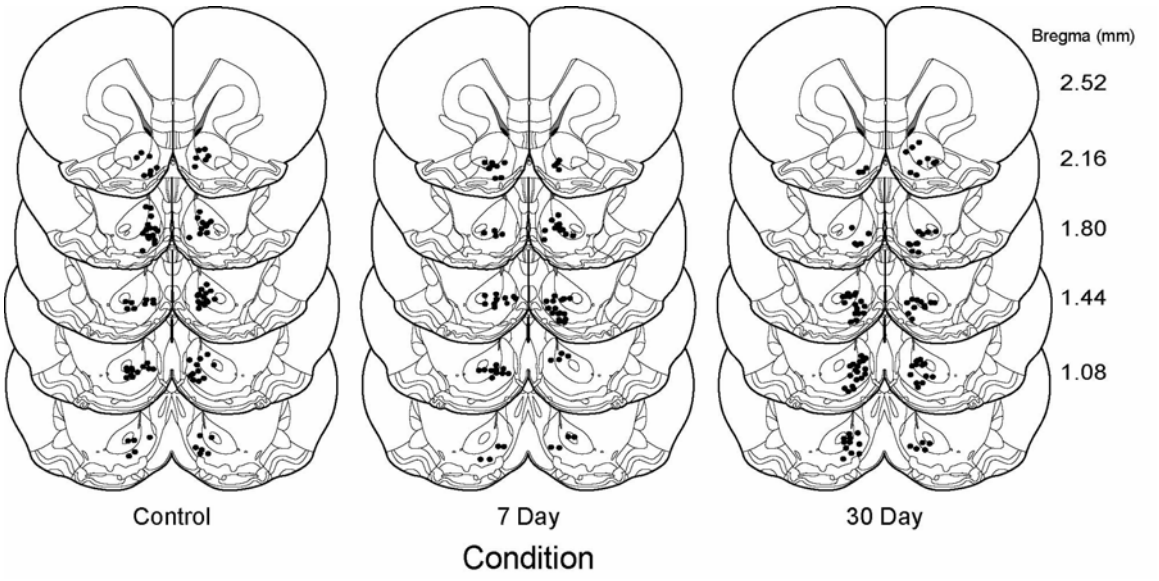


**Figure 5.** Anatomical distribution of electrode placements. Coronal sections showing locations of electrode tip placements from which cell firing was recorded within the core or shell of the NAc. Each abstinence condition is shown in its own histological series. Serial sections taken from Paxinos and Watson (1998).



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